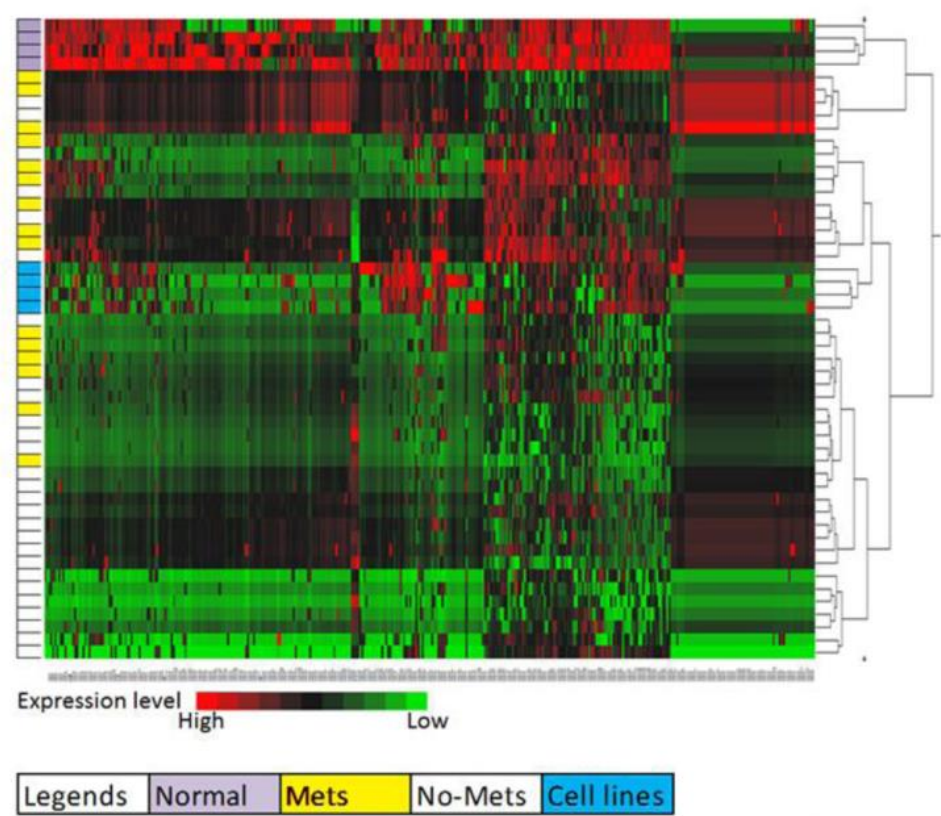


# Prognostic microRNAs modulate the RHO adhesion pathway: A potential therapeutic target in undifferentiated pleomorphic sarcomas

## Supplementary Material

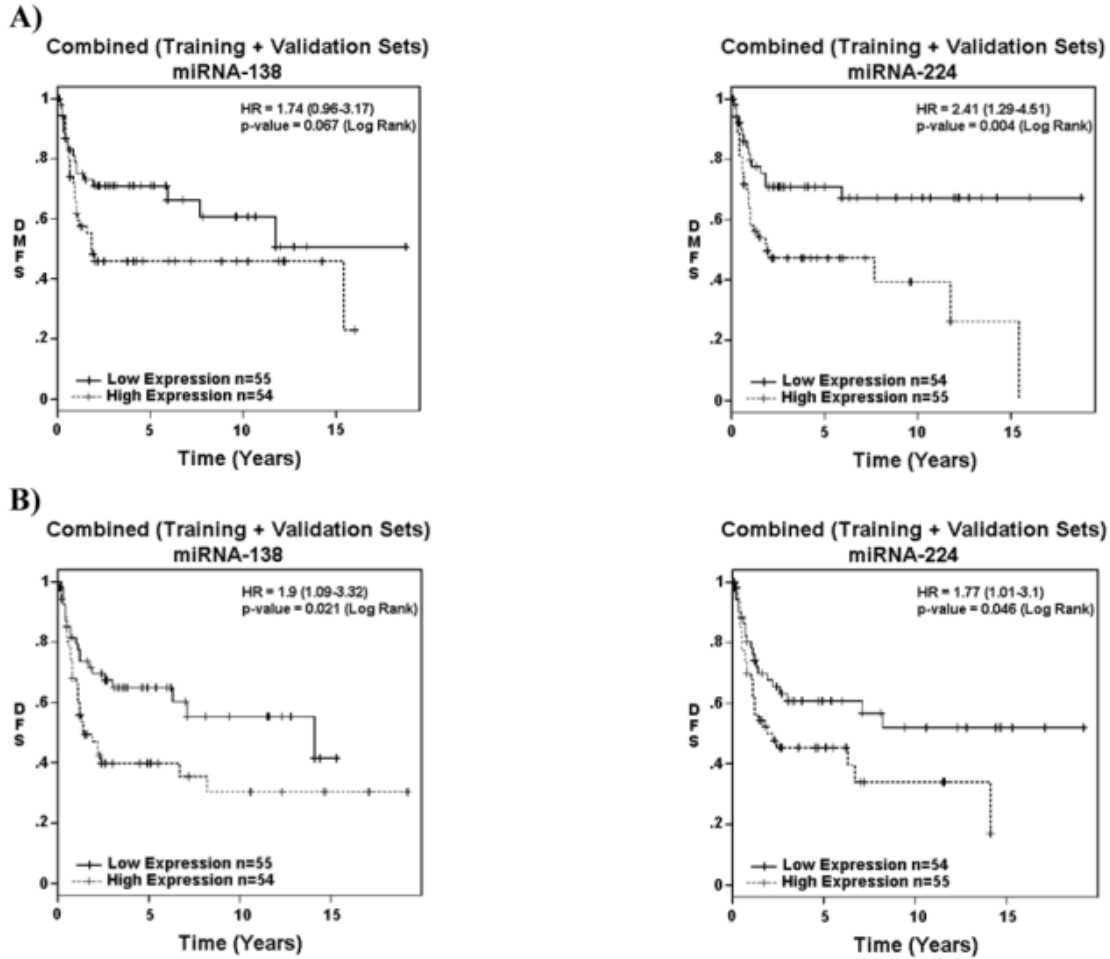
### SUPPLEMENTARY FIGURE 1

Unsupervised hierarchical clustering using the Ward method of miRNA expression from 4 Normal tissues (Adipose, Carotid, Vein and Smooth Muscle) (Purple), 4 primary UPS cell lines (STS48, STS93, STS109 and STS117) (Blue) and 42 primary UPS samples from the Training Set that metastasized (Yellow) or that did not-metastasize (White).



## SUPPLEMENTARY FIGURE 2

Univariate association of miR-138 and miR-224 expression with A) distant metastasis free survival (DMFS) and B) disease free survival (DFS) in the combined UPS cohorts (Training Set + Validation Set). C) Multivariate analysis of the associations of individual miRNAs from the 6-miRNA signature with DMFS and DFS in the combined UPS cohorts of Training Set and Validation Set. Combined datasets were used to obtain sufficient events for multivariate analyses. Values of miRNA expressions were dichotomized by the median  $\Delta C_t$  values. Multivariate adjustment was performed using 6 clinical factors (patient age, tumor size, grade, depth, gender and radiotherapy use).



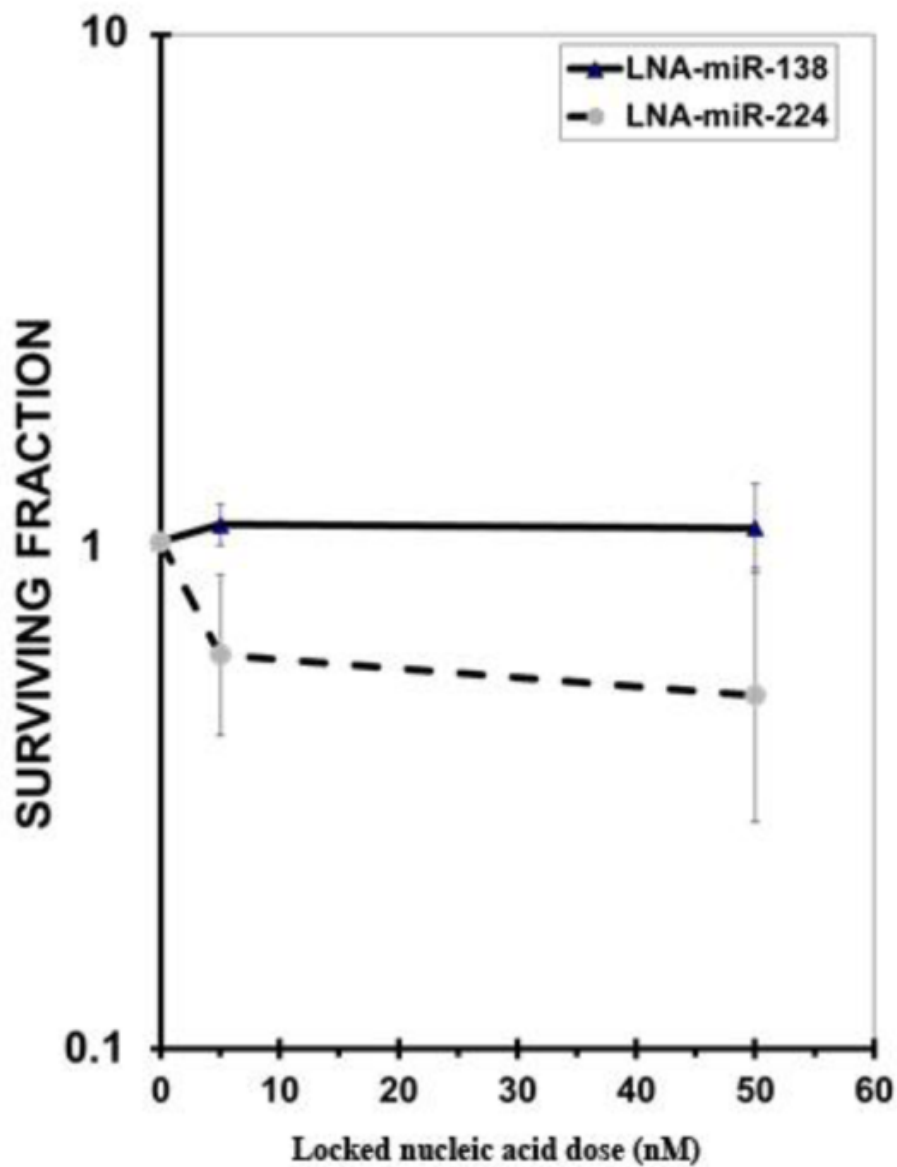
**C)**

		Multivariate Analysis for DMFS		Multivariate Analysis for DFS	
		Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Mir-132	Low vs. High	0.99 (0.51-1.9)	0.97	1.06 (0.58-1.94)	0.85
Mir-138	Low vs. High	2.62 (1.38-4.99)	<b>0.003</b>	2.47 (1.36-4.47)	<b>0.003</b>
Mir-143	Low vs. High	1.7 (0.91-3.2)	0.097	1.43 (0.81-2.54)	0.22
Mir-221	Low vs. High	1.17 (0.64-2.14)	0.62	1.08 (0.62-1.88)	0.78
Mir-224	Low vs. High	2.18 (1.12-4.21)	<b>0.021</b>	1.76 (0.98-3.18)	0.06
Mir-491-5p	Low vs. High	0.86 (0.45-1.63)	0.63	0.83 (0.46-1.49)	0.53

### SUPPLEMENTARY FIGURE 3

Clonogenic assay of STS117 cells following transfection with 5 nM and 50 nM of Locked Nucleic Acid (miR-138 and miR-224). Data are presented as the mean  $\pm$  standard error of the mean.

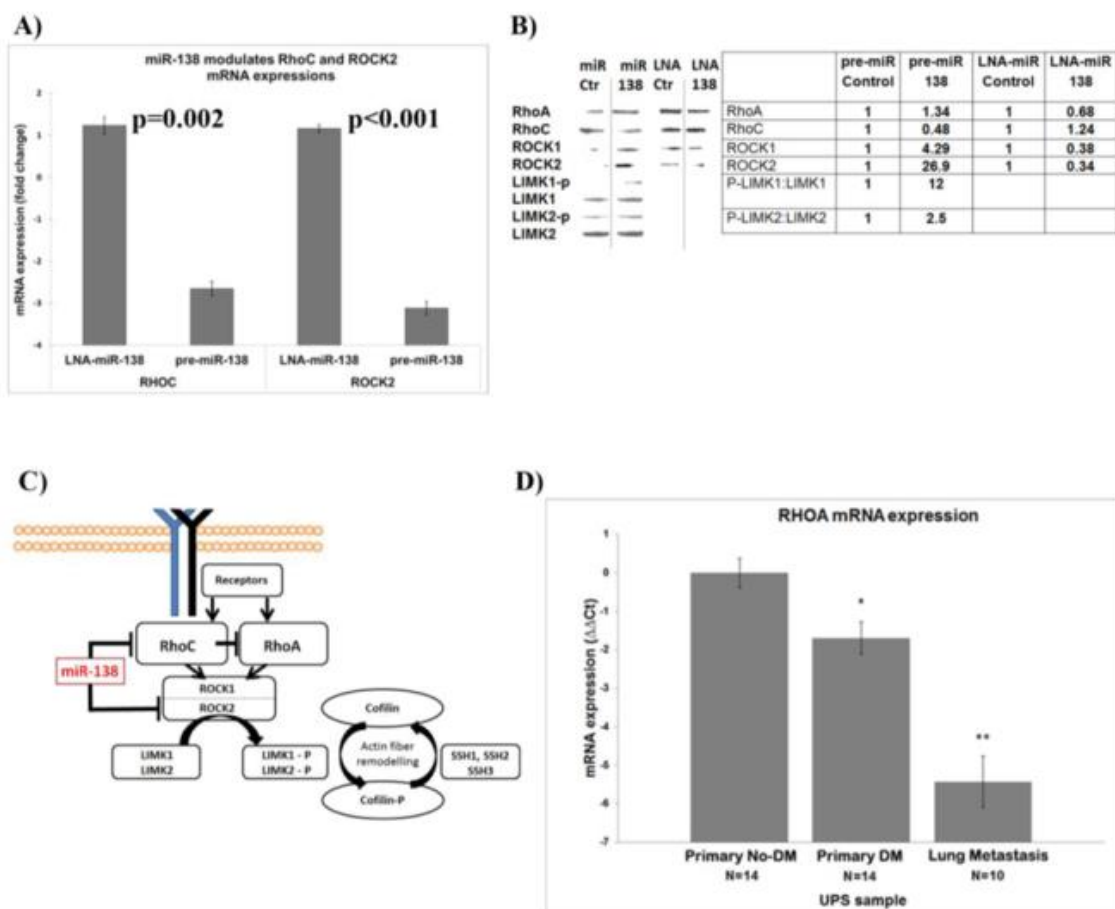
#### Clonogenic survival after miR modulation





## SUPPLEMENTARY FIGURE 4

Quantification in STS117 and STS48 of A) RhoC and ROCK2 mRNA levels following miR-138 modulation using pre-miR-138 or Locked nucleic acid (LNA) miR-138 demonstrating significant reduction in RhoC and ROCK2 expression with pre-miR-138 in relation to cells transfected with control (scrambled) pre-miR or LNA. B) Corresponding protein level and activity downstream of miR-138 and RhoA were assessed following transfection with pre-miR-control, pre-miR-138, LNA-miR-control and LNA-miR-138. C) A simplified Rho-ROCK-LIMK adhesion pathway depicts the targets of miR-138 (RhoC and ROCK2) and their interactions with RhoA and ROCK1. After binding with RhoA or RhoC, ROCK1 and ROCK2 can then activate the kinase activity of LIMK1 and LIMK2 through phosphorylation. The activity of Cofilin (1 and 2), a protein essential to actin fiber remodeling, is mediated by activated LIMKs and slingshot phosphatases (SSH1, SSH2 and SSH3). D) RhoA mRNA expression in primary UPS samples from patients who had no metastasis (Primary No DM) (n=14) vs. primary UPS which developed metastases (Primary DM) (n=14) vs. lung metastatic samples (Metastasis) (n=10). Significant differences in RhoA mRNA expression were observed between each group (Student t-test  $p \leq 0.006$ ). Data plotted as  $\Delta\Delta Ct$  normalized to the average values from the No-DM group (Lower values = Lower expression). MRNA expression data presented as the mean  $\pm$  standard error of the mean.

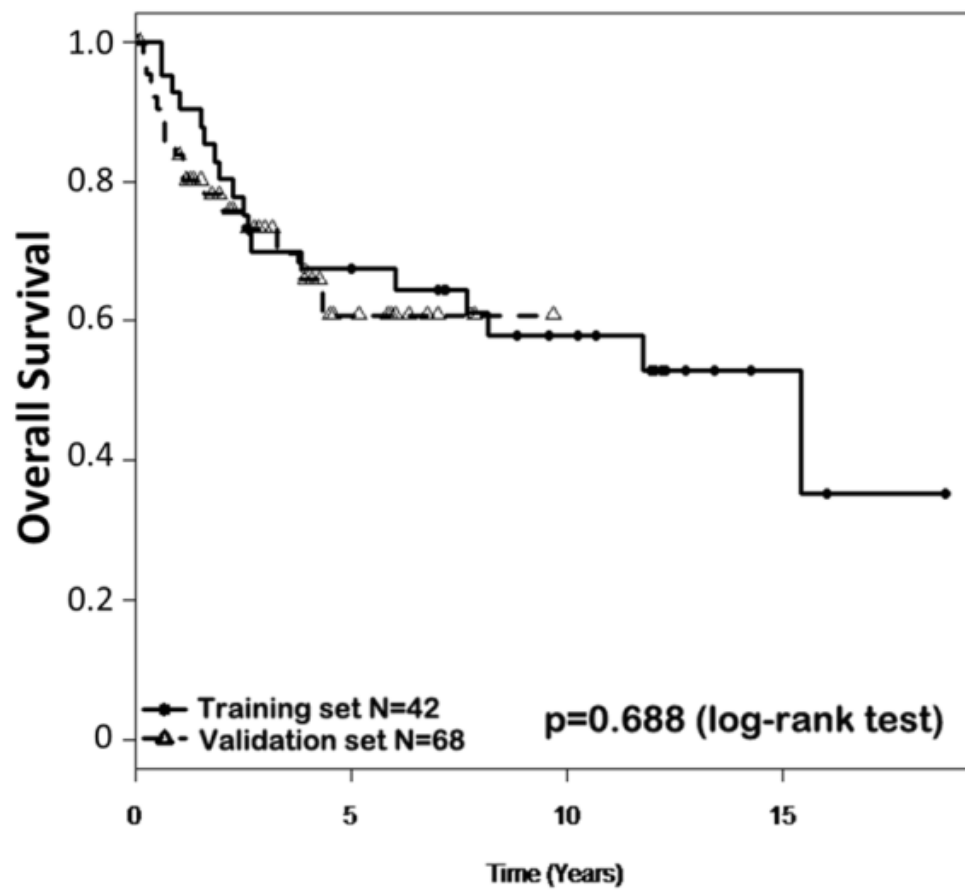


\*  $p=0.006$  (Primary No DM vs. Primary DM)

\*\*  $p<0.001$  (Primary No DM vs. Metastasis; Primary DM vs. Metastasis)

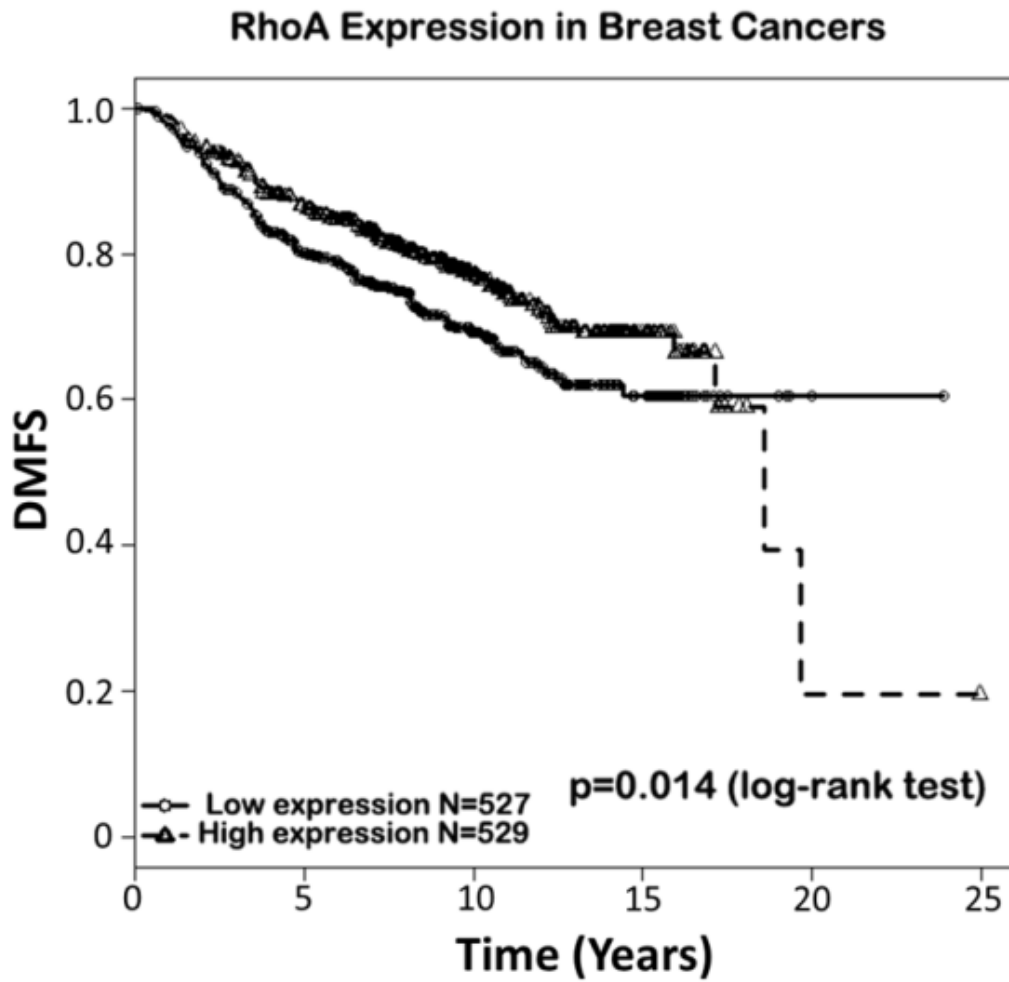
## SUPPLEMENTARY FIGURE 5

Overall survival of patients from the Training Set and Validation Set.



### SUPPLEMENTARY FIGURE 6

Distant metastasis free survival (DMFS) of 1056 breast cancer patients from the Combined breast dataset dichotomized by the median expression of RhoA. Median follow-up was 238 months.



**SUPPLEMENTARY TABLE 1: MiRNAs were associated with distant metastasis free survival**

Forty miRNAs were associated with distant metastasis free survival (DMFS) for the patients in the Training Set. These miRNAs are listed below according to the significance of the association (ascending p-value) with DMFS. Each miRNA's concordance index (C-index) with DMFS, difference in expression between samples that developed metastasis (DM) and those that did not metastasize (noDM), and its prognostic associations in cancers are listed. Thirty-one of the miRNAs potentially target 113 genes involved in the focal adhesion pathway (DIANA miRpath); the number of focal adhesion pathway genes potentially targeted by each miRNA is indicated in the last column and in the figure.

<b>miRNA</b>	<b>P-value</b>	<b>C-index</b>	<b><math>\Delta\Delta C_T</math> (DM – noDM)</b>	<b>Prognostic association with cancers (refs)</b>	<b>Number of genes in focal adhesion pathway</b>
miR-491-5p	0.003	0.702	1.786	Oral(1)	2
miR-224	0.004	0.686	2.298	Breast, Cervix, Colorectal, Germ cell tumor, Medulloblastoma Prostate, Pancreas(2-9)	8
miR-130a	0.005	0.677	0.400	Endometrial, Gastric, Lung(10-12)	16
miR-128	0.005	0.626	0.773		23
miR-886-5p	0.011	0.629	0.829		
miR-204	0.012	0.557	1.670	Melanoma(13)	6
miR-324-3p	0.013	0.648	0.582	Melanoma(14)	
miR-197	0.02	0.609	1.157	Breast, Lung(15, 16)	1
miR-99b	0.021	0.633	0.673	Esophageal(17)	
miR-744	0.024	0.665	1.036		1
miR-339-3p	0.027	0.642	0.389		
miR-512-3p	0.027	0.608	0.856		7
miR-15a	0.027	0.608	0.910	Larynx(18)	21
miR-23b	0.028	0.608	0.899		1
miR-202	0.028	0.619	1.297		7
miR-455-3p	0.029	0.654	0.892		
miR-200c	0.032	0.613	0.746	Colorectal, Esophageal, Hepatocellular, Melanoma(19-22)	1
miR-10a	0.034	0.641	0.388	Renal(23)	3
miR-139-5p	0.037	0.617	0.326		2
miR-132	0.037	0.613	0.492	Osteosarcoma(24)	1
miR-212	0.041	0.573	-0.04		10
miR-374a	0.041	0.641	0.508	Breast(25)	18
miR-486-3p	0.042	0.578	2.746		6
miR-148b	0.047	0.611	0.502	Breast, Pancreas(26, 27)	8





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**SUPPLEMENTARY TABLE 2: The association between breast cancer patients overall survival and the Rho pathway**

The association between TCGA BRCA patients overall survival (OS) with age, stage of disease and members of the Rho family (RHOA, RHOC, ROCK1, ROCK2, RHOBTB1, RHOBTB2, RHOBTB3, RHOB, RHOD, RHOF, RHOG, RHOH, RHOJ) and downstream ROCK-LIMK adhesion pathway (CFL1, CFL2, LIMK1, LIMK2, SSH1, SSH2, SSH3) was examined. Disease stage and 5 genes from the Rho-ROCK-LIMK family and pathway were associated ( $p < 0.05$ ) with OS of BRCA patients on univariate analysis. There is a high likelihood ( $p < 0.05$ ) that the 6-miRNA-signature score had interactive effects with 6 genes (RHOA, RHOBTB2, RHOC, RHOG, ROCK2, SSH3) from the cell adhesion pathway. From the 6 variables found to be associated with patient OS, 4 (RHOA, RHOBTB2, RHOC, RHOG) were highly likely to interact with the 6-miRNA-signature. Interaction analysis between BRCA tumor 6-miRNA-signature score with the expression of mRNAs from the Rho-ROCK-LIMK pathway, and estrogen receptor status was explored using Chi-Square tests.

<b>Variables analyzed</b>	<b>Correlation with OS (p-value)</b>	<b>Probability of interaction with 6-miR score (p-value)</b>
Age	0.08	<b>0.009</b>
Stage	<b>&lt;0.01</b>	0.771
ER status	0.06	<b>&lt;0.001</b>
LIMK1	0.1	0.435
RHOA	<b>0.03</b>	<b>0.021</b>
RHOBTB2	<b>0.04</b>	<b>0.012</b>
RHOC	<b>0.05</b>	<b>&lt;0.001</b>
RHOD	0.08	0.287
RHOG	<b>0.04</b>	<b>0.019</b>
ROCK2	0.08	<b>0.007</b>
SSH1	<b>0.05</b>	0.330
SSH3	0.07	<b>&lt;0.001</b>